PROTOCOL FOR INTRAPERITONEALLY IMPLANTED NIH: OVCAR-3

MODEL: (30C31) Intraperitoneally Implanted NIH: OVCAR-3 Human Ovarian Carcinoma

Origin of Tumor Line: (No details).

Summary of Test Procedures: Athymic random bred (NCr-nu) mice are implanted with one cc of 25% w/v OVCAR-3 cells. IP treatment begins day seven postimplant and is repeated every seventh day for three treatments. The parameter is median survival time. Results are expressed as a percentage of control survival time.

ANIMALS: (refer to Protocol 8).

Propagation and Testing: Athymic random bred (NCr-nu) mice.

Weight: Mice should have a minimum weight of 18 gm for males and 17 gm for females.

Age: Record age of mice.

Sex: One sex is used for all test and control animals in one experiment. Tentatively, females are preferred.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

EXPERIMENT SIZE:

General Testing: Eight animals per test group and 20 animals per control group.

TUMOR TRANSFER: (refer to Protocols 2, 5, and 6).

PROPAGATION

Implant: One cc of a 25% w/v cell suspensions, IP.

Time: When abdomen becomes distended (approximately Days 18-23 postimplant).

Site: IP

TESTING

Implant Preparation:

(1) Sacrifice donor mice and immerse in disinfectant.

(2) Attach mice to a cork board such that the skin covering the peritoneal cavity may be cut and teased away.

(3) Inject the peritoneal cavity with one or two cc of medium 199 or other physiological nutrient mixture.

(4) With a second syringe (5 cc with 18-gauge needle) withdraw as much ascitic fluid as possible. Steps three and four may be repeated. (The ascitic fluid will be very thick if not washed).

(5) Place the collected ascites in graduated centrifuge tubes (about 15 ml).

(6) Centrifuge in an International Clinical Centrifuge at 2000 RPM for five minutes.

(7) Pour off the supernatant.

(8) Determine the volume of packed cells and add three times that amount of medium 199 to resuspend the cells.

(9) Implant each animal IP with one cc.

TESTING SCHEDULE: (refer to Protocols 3 and 4).

Day 0: Make implant preparation and implant into the animals.

Day 1: Check cultures. Discard experiment if contaminated.

Day 7: Prepare test materials. Record body weights. Initiate IP test agent injections based on individual body weight. Treatment is q7d on days 7, 14, and 21. Prepare agent fresh on each injection day.

Day 14: Second treatment.

Day 21: Third treatment.

Day 23: Record body weights.

Evaluation Day: Depends on compound effectiveness. It may not be unreasonable to hold animals for 150 days if they show no clinical signs of disease after treatment. Terminating each group when the median day of death is reached is also an option.

QUALITY CONTROL: (refer to Protocol 7)

(1) Positive control compound is NSC 8806, 10 mg/kg/dose, day 7 only.

(2) Implant 2 or 3 additional mice which can be used for replacements in the event of unusual deaths prior to start of treatment. If unusual deaths do not occur, use these mice as additional control animals.

(3) Within a given experiment, whenever possible, use mice from the same supplier, date of receipt, and shipping crate to reduce fighting. If mice fight, house fighters individually.

(4) House mice according to AAALAC specifications.

(5) Donor tumor should be taken before the peritoneal cavity becomes filled with red blood.

(6) In case of unusual deaths, these animals should be autopsied and peculiarities noted.

(7) Include two additional control groups (8 animals each) that are implanted with 1:10 serial dilutions of the tumor preparation so that a tumor doubling time can be determined.

EVALUATION: (refer to Protocol 11).

The parameter measured will be median survival time. Compute mean animal body weights for Day 7 and Day 23, compute T/C for all test groups with >65% survivors on Day 23. (An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.) The expected median survival time will be about 50 days for the control group.

CRITERIA FOR ACTIVITY:

Not established.

REPORTING OF DATA:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request), and for poor suspensions.